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[Continued on next page]

(54) Title: OLIGONUCLEOTIDE FOR GENOTYPING OF MYCOPLASMA, MICROARRAY COMPRISING THE OLIGONUCLEOTIDE, AND METHOD FOR DETECTION OF SPECIES USING THE MICROARRAY

| MP-C [7] | <i>M. arginini</i> [28] | <i>M. arthrobidis</i> [30] | <i>M. fermentans</i> [39] | <i>M. hominis</i> [38] | <i>M. hyorhinis</i> [41] |
|-----------------------------------|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------|
| <i>M. neovaprophyllum</i> [49] | <i>M. opakescens</i> [52] | <i>M. orale</i> [58] | <i>M. pirum</i> [61] | <i>M. penetrans</i> [69] | <i>M. pulmonis</i> [75] |
| <i>M. salivarium</i> [63] | <i>M. coccaceae</i> [85] | <i>M. faecalis</i> [87] | <i>M. faecium</i> [90] | <i>M. hyosynoviae</i> [90] | <i>M. muris</i> [92] |
| <i>M. primatum</i> [96] | <i>M. spematophillum</i> [100] | <i>M. synoviae</i> [105] | <i>M. pneumoniae</i> [110] | <i>M. genitalium</i> [114] | <i>M. bovis</i> [120] |
| <i>U. urealyticum</i> [122] | | | AP-C [22] | <i>A. laidlawii</i> [128] | MP-C [7] |

*[] corresponds to SEQ ID No's of Tables 2 and 3.

(57) Abstract: The present invention relates to a method for detecting *Mycoplasma* and its related strains which are source of contamination of cell lines and biological products and human pathogenic. More particularly, the present invention relates to genus-specific and species-specific oligonucleotides for genotyping of *Mycoplasma*, *Acholeplasm* and *Ureaplasma* strains, microarray comprising the oligonucleotides, and method for detection of species using the microarray. As described above, the present invention provides a rapid and accurate assay method capable of simultaneously detecting many *Mycoplasma* and its related strains from a single sample using a microarray comprising novel oligonucleotides for detecting *Mycoplasma* and its related strains which are known as a source of contamination of cell lines and biological products and human pathogenic. Further, the present invention provides an objective and credible assay method capable of tracing a contamination source for preventing expansion of infective *Mycoplasma* and its related strains and controlling a contamination of *Mycoplasma* against biological products and stem cells or cord blood cells which are useful for gene therapy and cell therapy.

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**OLIGONUCLEOTIDE FOR GENOTYPING OF MYCOPLASMA,
MICROARRAY COMPRISING THE OLIGONUCLEOTIDE, AND
METHOD FOR DETECTION OF SPECIES USING THE MICROARRAY**

5 **Technical Field**

The present invention relates to a method for detecting *Mycoplasma* and its related strains which are a source of contamination of cell lines and biological products and human pathogens. More particularly, the present invention relates to genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma*, *Acholeplasm* and *Ureaplasma* strains, a microarray comprising the oligonucleotides, and a method for detecting strains using the microarray.

15 **Background Art**

Mycoplasma is a prokaryote pertaining to Mollicute family without cell wall, which was known as a hospital acquired pathogen causing pneumonia via infection of genital and respiratory organs of human as well as livestock such as pig and cow. Recently, *Mycoplasma* is more seriously understood as a major contaminant of cell culture and cell line

20 Especially, as the development and production of biological products for protecting and treating human diseases increases, the contamination of various pathogens provided by microorganism or clinical sample in the process of production became a serious problem. Examples of the biological products are an oncolytic virus, vaccine, a gene therapy vector and a recombinant protein. They have been found to be contaminated by bacteria, fungus, virus, *Mycoplasma* and its related strains (Doblhoff-Dier et al., 2001). The reason of the contamination is an organism contaminated in media components or experimental instruments and cross-contamination of microorganism and virus in air (Jung et al., 2003). Also, the contamination can be occurred

by a cross-contamination of already-infected WCB (Working Cell Bank) which is used for mass production of biological products (Wisher et al., 2002).

It is reported that, among these contamination sources, about 5 15~35% of cell culture or cell line is infected by *Mycoplasma* and its related strains (Hopert et al., 1993). This also makes experimental results incredible because it can change characteristics of cells such as abnormal synthesis of DNA, RNA and protein by binding to host cell wall 10 (Kong et al., 2001). As gene therapy and cell therapy are getting into the spotlight recently, an assay for infection of stem cell and cord blood by *Mycoplasma* and its related strain became more important. Therefore, for the credible and reproducible experimental results and the quality control of commercialized biological products, it is essential to detect an infection with *Mycoplasma* and its related strains.

15 Under this situation, Europe community make it a rule that, for credibility of safety and quality of food and drug, GMP (Good Manufacturing Practice) and QC (Quality Control) should be submitted and cell banks such as MCB (Master Cell Bank) and WCB should be subjected to an assay for detection of virus, fungus and bacteria such as 20 *Mycoplasma* (Doblhoff-Dier et al., 2001).

About 100 kinds of bacteria pertaining to Mollicute family without cell wall have been found so far, including *Acholeplasma*, *Enteroplasma*, *Mesoplasma*, *Mycoplasma*, *Ureaplasma* and *Spiroplasma*. Among them, about 20 kinds of *Mycoplasma*, *Acholeplasma* and *Ureaplasma* 25 are major contamination source of cell culture. These are referred to as “*Mycoplasma* and its related strains” in this specification. About 95% of the contaminants are covered by *M. arginini*, *M. fermentans*, *M. orale*, *M. hyorhinis*, *M. hominis*, *M. salivarium*, *M. pirum*, *A. laidlawii* (Dorigo-zetsma et al., 1997). However, *Mycoplasma* is difficult to be cultured in 30 extracellular media and turbidity is rare in the culture. Therefore, there has been a need to the rapid and accurate genotypic detection method

which can trace a contamination source of *Mycoplasma* and its related strains.

Conventional *Mycoplasma* detection methods are the culturing method, the DNA fluorochrome stain method, the immunofluorescence method, and the polymerase chain reaction (PCR) method (Dorigo-zetsma et al., 1997). However, the culturing method has a drawback that extracellular culturing is difficult, preparing its media is complex by adding supplements such as serum and culturing time is too long, about 4 days ~ 3 weeks according to the kinds of strains (Jensen et al., 2003).

The DNA fluorochrome stain method such as Hoechest 33258 stain has a drawback that culturing condition is too difficult to match and subjective inspectors can make a misjudgment (Chen et al., 1997). The immunofluorescence method such as ELISA has a drawback that bacteria having similar antigen with *Mycoplasma* such as *Streptococcus milleri group* and *Staphylococcus aureus* may raise a false positive signal due to of low specificity (Hopert et al., 1993). The PCR method makes use of 16S/23S intergenic spacer region (ITS) and a gene coding 169 kDa of P1 cyadhesion proteine which represent variety of *Mycoplasma* (Uphoff et al., 2002). The P1 gene, a surface antigen gene, has several subtypes representing diversity and has been used as a target gene for serological detection using immune reaction and genotypic detection using restriction fragment length polymorphism (RFLP) to identify *Mycoplasma* (Campo et al., 1998). However, most of conventional PCR methods use a primer designed based on 16S rRNA which is a common sequence of prokaryotes, and second PCR or nested PCR having high sensitivity can make a cross-contamination of *Mycoplasma* dispersed in air and an amplification of a bacteria similar with *Mycoplasma* in classification (Uphoff et al., 2002).

To overcome the above limitations of the conventional detection methods, a genotypic detection method using probes have been developed recently, which make it possible to analyze many kinds of

genes in a short time using DNA hybridization principle based on gene sequencing and detect specifically a single base change using a proper hybridization condition between specific probe and target DNA.

The present inventors developed ITS-derived oligonucleotides capable of detecting *Mycoplasma* and its related strains, which are important in genotypic detection, and a microarray comprising the oligonucleotides as a probe for detecting *Mycoplasma* and its related strains.

10 **Disclosure of the Invention**

It is a first object of the present invention to provide oligonucleotides for detecting *Mycoplasma* and its related strains designed based on their ITS base sequences.

15 It is another object of the present invention to provide novel ITS sequences of *Mycoplasma bovis*, *Mycoplasma cloacale*, *Mycoplasma falconis*, *Mycoplasma faecium*, *Mycoplasma spermophilum* and *Mycoplasma synoviae*, which is useful for detecting *Mycoplasma* and its related strains.

20 It is another object of the present invention to provide a microarray comprising genus-specific and species-specific oligonucleotides for detecting *Mycoplasma* and its related strains as probes.

It is another object of the present invention to provide a method for detecting *Mycoplasma* and its related strains using the microarray.

25 It is another object of the present invention to provide a kit for diagnosing *Mycoplasma* and its related species infection individually or simultaneously, comprising genus-specific and species-specific oligonucleotides for genotyping *Acholeplasma* and related strains.

30 According to an aspect of the present invention, there is provided a purified ITS (internal transcribed spacer) target DNA for genotyping

Mycoplasma strains, comprising any one sequence selected from SEQ ID Nos. 1 to 6.

SIQ ID Nos. 1 to 6 are base sequences of ITS (internal transcribed spacer) of *Mycoplasma bovis*, *Mycoplasma cloacale*, 5 *Mycoplasma falconis*, *Mycoplasma faecium*, *Mycoplasma spermophilum* and *Mycoplasma synoviae*, which was newly obtained by base sequencing analysis.

The ITS target DNA of the present invention can be used indirectly for designing probes or primers used for genotyping 10 *Mycoplasma* strains or directly for genotyping *Mycoplasma* strains via PCR amplification.

According to another aspect of the present invention, there is provided an oligonucleotide for genus-specific genotyping of 15 *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 7 to 21 or its complementary sequence.

According to another aspect of the present invention, there is provided an oligonucleotide for genus-specific genotyping of 20 *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 22 to 27 or its complementary sequence.

According to another aspect of the present invention, there is provided an oligonucleotide for species-specific genotyping of 25 *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 28 to 127 or its complementary sequence.

According to another aspect of the present invention, there is provided an oligonucleotide for species-specific genotyping of 30 *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 128 to 133 or its complementary sequence.

The oligonucleotides according to the present invention are designed based on multiple sequence alignment of ITS (internal transcribed spacer) sequences, which are present between 16S rRNA 35 and 23S rRNA of *Mycoplasma* and its related species. The

oligonucleotides can be used as primers for PCR amplification in order to genotype *Mycoplasma* and its related species or as probes for hybridization reaction in order to genotype *Mycoplasma* and its related species.

5 According to another aspect of the present invention, there is provided a microarray comprising more than one oligonucleotides selected from genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma*, *Acholeplasma* and *Ureaplasma* strains according to any one from claims 2 to 5 as probes attached on a support.

10 In the microarray according to the present invention, the probes may be any materials having base sequence, preferably any one selected from a group consisting of DNA (Deoxyribose Nucleic acid), RNA (Ribose Nucleic Acid), and nucleic acid analogues such as PNA (Peptide Nucleic Acid), LNA (Locked Nucleic Acid) and HNA (Hexitol Nucleic Acid).

15 In the microarray according to the present invention, the support may be any materials to which the probes can be attached, preferably any one selected from a group consisting of slide glass, plastic, membrane, semiconductive chip, silicon and gel. The microarray according to the present invention can be manufactured using conventional method such as pin microarray, ink jet, photolithography or electric array method.

20 The microarray according to the present invention can be used for simultaneously genotyping various *Mycoplasma* and its related species which are known as a major contaminant of biological drug and cell line as well as a human pathogen from one sample, as the microarray comprises genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma* and its related species as a set attached a support

25 30 According to another aspect of the present invention, there is provided a method for detecting *Mycoplasma*, *Acholeplasma* and

Ureaplasma strains, comprising the following steps:

- a) extracting nucleic acids from a sample;
- b) amplifying target DNA among the extracted nucleic acids;
- c) hybridizing the amplified target DNA with probes of the microarray according to the above present invention; and
- d) detecting signals generated from the hybridization reaction.

In the detection method according to the present invention, the sample may be biological drug, cell line, or human tissues or serum. The purifying step can be performed using conventional DNA or RNA purification method or kit. The signal detecting step can be performed using a conventional fluorescence scanner after binding conventional fluorescent dyes such as Cy5 or Cy3.

According to another aspect of the present invention, there is provided a kit for diagnosing *Mycoplasma* and its related species infection, comprising more than one oligonucleotide selected from genus-specific and species-specific oligonucleotides for genotyping *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains according to the above present invention.

In the kit according to the present invention, the oligonucleotides are used as probes for hybridizing with target sample and may be contained in a proper vessel. The probes may be labeled with a radioactive or non-radioactive labeling agent, the latter comprises conventional biotin, Dig(digoxigenin), FRET(fluorescence resonance energy transfer) or fluorescent dye (Cy5 or Cy3). Further, the oligonucleotides can be used as primers for PCR amplification. In this case, the kit may contain DNA polymerase, 4 dNTPs and PCR buffer for PCR reaction. In addition, the oligonucleotides can be attached to a microarray as probes. In this case, the kit may contain hybridization reaction buffer, PCR kit containing primers for amplifying a target gene, washing solution for the unhybridized DNA, dyes, washing solution for unbound dyes and manual sheet for the microarray.

Hereafter, the present invention will be described in more detail.

The present invention provides a method for detecting or genotyping *Mycoplasma* and its related strains which is a major contamination source of cell lines and biological products and a human pathogen, comprising the following steps:

- 5 a) if necessary, extracting nucleic acids from a sample such as cell lines, biological products or human tissue or serum;
- 10 b) if necessary, amplifying target DNA of *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains among the extracted nucleic acids using more than one proper primers;
- 15 c) hybridizing the amplified target DNA with probes having a sense or antisense or complementary sequences of genus-specific and species-specific oligonucleotides of *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains disclosed in Tables 2 and 3; and
- 20 d) detecting signals generated from the hybridization reaction.

From the detected signals in the step d), the existence of *Mycoplasma* and its related strains in the sample can be predicted.

The present inventors carried out a sequence analysis of ITS regions of many *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains to obtain genus-specific and species-specific oligonucleotides for detecting *Mycoplasma* and its related stains which can be a basis of developing a specific and sensitive hybridization assay. Also, the present inventors newly analyzed ITS sequences of newly found 6 *Mycoplasma* strains, which makes it possible to design probes capable of detecting more various *Mycoplasma* and its related strains.

Table 1 discloses ITS sequences of newly analyzed 6 strains among target sequences for detecting *Mycoplasma* strains, which correspond to SEQ ID Nos. 1 to 6. In the present invention, the probes for detecting *Mycoplasma* strains were designed based on the multiple alignment of ITS sequences of *Mycoplasma*.

FIGS. 1 and 2 show multiple sequence alignments of ITS regions

of *Mycoplasma*, *Acholeplasma* and *Ureaplasma* for selecting genus-specific and species-specific probes of *Mycoplasma* and its related strains. Genus-specific oligonucleotides of *Mycoplasma* and *Ureaplasma* were designed from conservative sequence region indicated by a box in FIGS. 1a to 1f. Species-specific oligonucleotides of *Mycoplasma* and *Ureaplasma* were designed from polymorphic sequence region outside the box in FIGS. 1a to 1f. Genus-specific oligonucleotides of *Acholeplasma* were designed from conservative sequence region indicated by a box in FIGS. 2a to 2c. Species-specific oligonucleotides of *Acholeplasma* were designed from polymorphic sequence region outside the box in FIGS. 2a to 2c.

In step b) of the present invention, the target DNA of *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains were amplified using more than one pair of proper primers. FIG. 3 shows PCR amplification of ITS target sequences of *Mycoplasma* and its related strains using a primer pair, MP16SF-2 and MP23SR-2. In FIG. 3, 1 is a PCR product of *M. arginini*, 2 is a PCR product of *M. arthritidis*, 3 is a PCR product of *M. fermentans*, 4 is a PCR product of *M. hominis*, 5 is a PCR product of *M. hyorhinis*, 6 is a PCR product of *M. neurolyticum*, 7 is a PCR product of *M. opalescens*, 8 is a PCR product of *M. orale*, 9 is a PCR product of *M. pirum*, 10 is a PCR product of *M. penetrans*, 11 is a PCR product of *M. pulmonis*, 12 is a PCR product of *M. salivarium*, 13 is a PCR product of *M. cloacale*, 14 is a PCR product of *M. falconis*, 15 is a PCR product of *M. faecium*, 16 is a PCR product of *M. hyosynoviae*, 17 is a PCR product of *M. muris*, 18 is a PCR product of *M. primatum*, 19 is a PCR product of *M. spermatophilum*, 20 is a PCR product of *M. synoviae*, 21 is a PCR product of *M. pneumoniae*, 22 is a PCR product of *M. genitalium*, 23 is a PCR product of *M. bovis*, 24 is a PCR product of *U. urealyticum*, 25 is a PCR product of *A. laidlawii*.

In step c) of the present invention, the amplified target DNA were hybridized with probes for detecting *Mycoplasma* and its related strains.

Preferably, the probes may be a combination of more than one probes capable of simultaneously detecting many *Mycoplasma* and its related strains from a single sample. Practically, the probes are optimized to simultaneously hybridize with multiple target DNAs of *Mycoplasma* and its related strains under the same hybridization and washing conditions.

The present invention provides a microarray comprising a set of probes for detecting *Mycoplasma* and its related strains, which can simultaneously detect many *Mycoplasma* and its related strains from a single sample with a single experiment.

In the present invention, the term 'probe' means a single-stranded oligonucleotide having a sequence complementary to target DNA of *Mycoplasma*, *Acholeplasma* and *Ureaplasma*. The probe may have a sense, antisense or complementary sequence of SEQ ID Nos. disclosed in this specification as long as it can hybridize with one of double strands of target DNA. The oligonucleotide may be ribonucleotide (RNA), deoxynucleotide (DNA), peptide nucleic acid (PNA) or locked nucleic acid (LNA), and contain modified nucleotides such as Inosine only if it does not change their hybridization characteristics. Preferably, the genus-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* may have a base sequence of SEQ ID Nos. 7 to 27. Preferably, the species-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* may have a base sequence of SEQ ID Nos. 28 to 133.

FIG. 4 shows a microarray comprising probes for detecting genotypes of *Mycoplasma* and its related strains as a set on a support. In FIG. 4, each species name and SEQ IN Nos. are described which correspond to individual probes. The terms 'MP-C' and 'AP-C' mean *Mycoplasma* and *Ureaplasma* genus and *Acholeplasma* genus. FIG. 4 is no more than an example of probe compartment of the present invention, so compartment and layout of each probe can be varied.

In the present invention, newly analyzed ITS sequences of 6

Mycoplasma strains as a target DNA for detecting *Mycoplasma* and its related strains are as shown in Table 1. The genus-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* used in the present invention are as shown in Table 2. The species-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* used in the present invention are as shown in Table 3.

【Table 1】

| Species | Sequence (5' → 3') | SEQ ID NO. |
|----------------------|--|------------|
| <i>M. bovis</i> | TTC TACGG AGTAC ACTTG TCTTTA TCACTATAAAAAAG ACTTATAAOCAAAT TACTAGACCTATA TTTTATTATAAAACG TCA TGCGTTTTATTAA TAGG TCAAAGCTA TATACTAGTTTGAGAGAACATTC TC TCA TATG TIC TTG AAAACTGAATAGTAAA ATATTTTCGATA TTTACAACGACATCAAAAACTAAATTAAAGGTTAATTTGTTTG ATTCA TCGAGTAAGTCATA TTTAATAATGATC ATGAAATGCTTTAAACATACACATC TAAAACTAACAAACATAGGAAAATACTACTTTTAAATAAGGAAGAGTTTGGTGG ATGC | 1 |
| <i>M. caviae</i> | CTTC TACGG AGTAC AA TCT CACTG TTATGGAATTAATTTG TATCCAGTTTGAGA GAACTTCTCTCAATTTTTGTTCTTGAAAATCTGAATATAGACATGAAATCAATAAA TTAATTTCAAAATGTTAGATCAACCTATAGAAATTTCAAGACATATAACAAAATA GGTCA TACTTATA TTTATAAATCT | 2 |
| <i>M. felis</i> | CTTC TACGG AGTAC AACTCTGTTATGGAATTAATTTG TATCCAGTTTGAGAT ACTAACTCTCTTCTGTTCTTGAAAATCTGAATATCGACATGAAAATTTAAATT AATTTCAAAAGTTAGATCAACCTATAGAAATACAAAATA TAGACAACAAATAGGT CATAACACAAATAACAAAACACT | 3 |
| <i>M. leucomii</i> | GAATGGTGGCTCTGGAGACTAAAGTTATGGAAAACATCG TATCCAGTTTGAGA GAACTAAACTCTCTCTTTGTTCTTGAAAATCTGAATATAGACATGAAAATTTAAAA AATTTAAATTTCIAAGTTAGATCAACCTATAGAAATACAAAATA CAATACAAATAGG TCAATAC TATACAATTGCATAACAAAATA TACTTTAAACAAAGATAAGAGTTTG GTGGATGCAATTGTA | 4 |
| <i>M. pneumoniae</i> | GTTGGGGATGGATCACCTCTTCTACGGAGTACAAACATACATCAAAATTTGACT GAATGTTAATACCTTATTTTCTACTAGGGCTTTTTAATAATTTGTTGTTGACT TTATGGCCTAAAGCTTATACTAGTTTGAGAGACATCCCTCTCAATTGTTCT TTGAAAATCTGAATAGTAAATTTTGATAATTTACAACGACATCTAAATAATIGAAAT AAGTCATTTGTTAGATCTACGAGATGTCATTITAAAAAAATGATCATGAAATGTCCTTAAATAACACATCAAAACAAACAAATCTATACAAATAGGAAATTATATAACT | 5 |
| <i>M. synoviae</i> | TCC TACGG AGTAC ATTAATTTACAAAGGCTTTTATTAATCTGAAGCTTTAG AGAAAAAAATCTAAAAGCGGTTGTGATCGCTTTTTGCTTGGGCTATTGTTTA GTTTGAGAGAACACCTCTCTTAAATTTGTTCTTTGAAAATCTAAATAAGGTTAATTTGTTGATACOGAGTTAAATTATGAAATAATAATTATTTAAATGTCCTTGAATACATCATAACAAATAAACAATAGGACATATTGATACTTAACCTTTAAAAAGT | 6 |

| Genus | Probe | Sequence | SEQ ID NO. |
|--|--|--|----------------------------------|
| <i>Mycoplasma</i> | MP-CP1 MP-CP2 | TCTTTGAAAACGTGA RWTCCTTVAAAACTTATWN | 7 8 |
| <i>M. arginini</i> , <i>M. arthritidis</i> <i>M. caviae</i> , <i>M. falconis</i> <i>M. faecium</i> , <i>M. hominis</i> <i>M. hyosynoviae</i> , <i>M. orale</i> <i>M. salivarium</i> | MP-CA1 MP-CA2 | MWTYG TTTCCAG TTTTGAGAG TTTAAG ATCAACCTATAGAATA | 9 10 |
| <i>M. bovis</i> , <i>M. fermentans</i> <i>M. opalescens</i> , <i>M. primatum</i> , <i>M. spermatoaphilum</i> , <i>M. synoviae</i> | MP-CB1 MP-CB2 MP-CB3 MP-CB4 | TTATYTAG TTTTGAGAGGTTCA WWTRATTYATTTAAATG TCTT GG KYAATTIG TTTWGAT TTATTTTACAMCG MCAYC | 11 12 13 14 |
| <i>M. marinis</i> , <i>M. penetrans</i> <i>M. urealyticum</i> | MP-CC1 MP-CC2 | CC TCC TTCTATCGGAGTAMA CGGA TTCTATTAG TTTTGAG | 15 16 |
| <i>M. neurolyticum</i> , <i>M. pulmonis</i> | MP-CD1 MP-CD2 MP-CD3 | TAAAATAGATACCTTAAKATA GTATYYAG TTTTGAAAG CTTGCAAWTAGWTWT | 17 18 19 |
| <i>M. genitalium</i> , <i>M. pneumoniae</i> | MP-CE1 MP-CE2 | AWACRACAATCTTTCTAGTTC AATAAGTTACTAAGGGCTTAT | 20 21 |
| <i>Acholeplasma</i> | AP-CP1 AP-CA1 AP-CA2 AP-CA3 AP-CB1 AP-CB2 | TCAATCATATTICAGTTTG GGGCCTTTAGCTCAGYTGG TT AGAGCRCWCGCYTGATAAGCG WGRGGTCGATGGGTTCAGTCC TCATCATATTICAGTTTGARR AGCTTTGAAAAGTAGATAAA | 22 23 24 25 26 27 |

【Table 3】

| Species | Probe | Sequence | SEQ ID NO. |
|-------------------------------|--|--|--|
| <i>M. arginini</i> | MP-arg1 MP-arg2 | AGATTATAATCATAACATAGA GAGTACATAAAATGGTTATGGAA | 28 29 |
| <i>M. arthritidis-faecium</i> | MP-ari1 MP-ari2 MP-ari3 | TGAAGGCCGATGGTGCTTCG TGAGAGAACTAACTTCCTC GAATACAAAAATCAATACAATA | 30 31 32 |
| <i>M. fermentans</i> | MP-fer1 MP-fer2 MP-fer3 MP-fer4 MP-fer5 | ATG TACTTTAACCTTATTCAC TACAAAAGAGTACTTTTTAAA TTTTATGGGTCTAAAGCTTT GAACAATATTTTTCTCTCA ATAACAAACTATAACAATAAGG | 33 34 35 36 37 |
| <i>M. hominis</i> | MP-hom1 MP-hom2 MP-hom3 | ATTTATCTCTCGGTTCTTT ATATTATATTTTATAAGACA ATGATAATAATTAAATATT | 38 39 40 |
| <i>M. hyorhinis</i> | MP-hyo1 MP-hyo2 MP-hyo3 MP-hyo4 MP-hyo5 MP-hyo6 MP-hyo7 MP-hyo8 | GAATAGCAGATAACAAATATGATT CGGAGTACATTAGCTTAATT TTACATAATCGATTCGTTGCT AGCTTTAAGTTCTCAATTTAA TICATATTIATTATTTCACG AACGAATCTTTTATAACCGA TTAAATTCCTAAATAGATTA AGATATTAACTTTAGCAATA | 41 42 43 44 45 46 47 48 |
| <i>M. neurolyticum</i> | MP-neu1 MP-neu2 MP-neu3 | GGTTATTATGGGCTTGCTA GGTTATTAAATAATCCTTTA TAATTTTCTCTCTTAATTAA | 49 50 51 |
| <i>M. opalescens</i> | MP-opa1 MP-opa2 MP-opa3 MP-opa4 MP-opa5 MP-opa6 | CATCATAATGTAACCAATAC ACAAAAATCATTATTTTAAT TTAAATGATTAATTAACCTTT TTAATGCTTGTCTTTATGG TATGGTCTACAAAAGCTTATAT GATAAAAAACAACTCATAAATT | 52 53 54 55 56 57 |
| <i>M. oralis</i> | MP-ora1 MP-ora2 MP-ora3 | CATAAAATAGTTAATGGCICA ATAGAGACAAATACAAAAACAA GGTCAAAAATACCTTATACGTA | 58 59 60 |
| <i>M. piruri</i> | MP-pir1 MP-pir2 MP-pir3 MP-pir4 MP-pir5 | TAGTTCTTIGTGTAATAACA CTTTATACACCTTATACAAAT TAAATCTCAATTAAATGTAA GCAAATTGATGTCACACATT AATTAATCTCTCTTATTACTT | 61 62 63 64 65 |

| | | | |
|---------------------|--|--|--|
| | MP-pir6 MP-pir7 MP-pir8 | TTAAAGTAGTAGAGATGGTTC CAAATATCAAATGCTAAIGGA ATGCTAATGGATATCAAAAAAA | 66 67 68 |
| <i>M. penetrans</i> | MP-pen1 MP-pen2 MP-pen3 MP-pen4 MP-pen5 MP-pen6 | AAGAGTAAGTCTCTAGGTGCG CATTAAGCTAAGTAACAAAT TCCTAAACTGAATAATTATCT TTATAAAGAGTAAGTCTCTAG ATTTTCCTCTCAAGATAGTTC TCATAATCATACTTGTTATTT | 69 70 71 72 73 74 |
| | MP-pui1 MP-pui2 MP-pui3 MP-pui4 MP-pui5 MP-pui6 MP-pui7 MP-pui8 | AATTTTTGATCCGAGTCATTC CATTTTCTATCAATAGTTAT TAATGTTATCTTGGCCAATTAG TTCTATCTTCAAAACAAATA TATAAATTAAATATGATAACGT TCATCAAAATGTTAAAATTTT AAAAATAAAATAGATACCTTA AAATAAATTCAACAAATAGGA | 75 76 77 78 79 80 81 82 |
| | MP-sal1 MP-sal2 | TAATGGATTTAATTTTCGTTG TATCAAAATCAATATAATTTT | 83 84 |
| | MP-cl01 MP-cl02 | AGTACAACTCTCACTGTTATG TAGAATAATCAAGACATATAC | 85 86 |
| | MP-fal1 MP-fal2 MP-fal3 | GAGTACAACACTCTGTTATG AGAATACAAAAATATAGACAA ATTGAAAAATTATAATTAAAT | 87 88 89 |
| | MP-hyos1 MP-hyos2 | CTAGACTAAAGTTATGGTAC AATTATCAAATTAAATATTCA | 90 91 |
| <i>M. muris</i> | MP-mur1 MP-mur2 MP-mur3 MP-mur4 | TATAGAAAACCCACATCA TTTGAATAATTAAATATT GATTATTACACCATTAGAA TCAATAAACCTAAATAAAAAAA | 92 93 94 95 |
| | MP-pri1 MP-pri2 MP-pri3 MP-pri4 | GTAGACATAACCCCAAGCTA CAAACGTCATAAGCTTTTAG TCATGGGCTTTAATAGGGTC ACCCCCAACCTCCCACATCAAAAT | 96 97 98 99 |
| | MP-spe1 MP-spe2 MP-spe3 MP-spe4 MP-spe5 | TTCATCGAGATAGTCATTTA CAAACATACATTCAAAATTIT TTTGACTGAATGTTATTAAAC TTTGTATG TGACTTTTATGG AAAACAAACAATCTATACAAAT | 100 101 102 103 104 |

| | | | |
|-----------------------|---------|-------------------------|-----|
| <i>M. synoviae</i> | MP-syn1 | TGGCTTGGGCTATTGTATT | 105 |
| | MP-syn2 | GCGGTGGTGATAGCTTTT- | 106 |
| | MP-syn3 | ACCTCTCTAAATATGTTCTT | 107 |
| | MP-syn4 | CCGAGTTAATTATTGAAATA | 108 |
| | MP-syn5 | CATCATAACAACAATAACAA | 109 |
| <i>M. pneumoniae</i> | MP-pne1 | GTAATTAAACCCAAATCCC | 110 |
| | MP-pne2 | ATCTTAATAAAAGATAATAC | 111 |
| | MP-pne3 | CTAACACAAAATCAAAATCC | 112 |
| | MP-pne4 | AAAGAACATTCCGCCTCTT | 113 |
| <i>M. genitalium</i> | MP-gen1 | CACCCCTTAATTTCGCG | 114 |
| | MP-gen2 | AATGGAGTTTTATTTTTTTA | 115 |
| | MP-gen3 | CCCAAATCAATGTTGGTC | 116 |
| | MP-gen4 | CAACTAACACACTTGGTCAGT | 117 |
| | MP-gen5 | AGAAATGTTTTGAAACAGTC | 118 |
| | MP-gen6 | TAGTICCAAAAAATAAAATAC | 119 |
| <i>M. bovis</i> | MP-bov1 | TATAACCAAAAATAAAGACCTA | 120 |
| | MP-bov2 | GTCATGGCTTTTATAATTAGG | 121 |
| <i>U. urealyticum</i> | UP-ure1 | CATTAAGTTGTCAGTGAA | 122 |
| | UP-ure2 | TAATTTACGTTACTAATAAGTG | 123 |
| | UP-ure3 | TTTATTTAAAATCCATATAGAAT | 124 |
| | UP-ure4 | AAGCCACTTTTTAAAAATT | 125 |
| | UP-ure5 | CCATAATAATTAAATTATAT | 126 |
| | UP-ure6 | ATTATCAACAAATCTTCTAA | 127 |
| <i>A. laidlawii</i> | AP-lai1 | AACACTTGTGACAAGATGAC | 128 |
| | AP-lai2 | CTTCTTAAGGAGAAAGGCTAA | 129 |
| | AP-lai3 | ATGACTACTAGTTAAGTAGTAA | 130 |
| | AP-lai4 | GTAGTAATATTCTCTAAATT | 131 |
| | AP-lai5 | TTAAAGTAATTAAAGTGTTTC | 132 |
| | AP-lai6 | TAAATGATGTCGAAAAAGAAA | 133 |

* Mixed Base의 Code Name

M : A + C, W : A + T, Y : C + T, R : A + G
 K : G + T, V : G + A + C, N : A + G + C + T

5

Brief Description of the Drawings

FIGS. 1a to 1f show multiple sequence alignments of each ITS region of *Mycoplasma* and *Ureaplasma* for selecting genus-specific probes.

FIGS. 2a to 2c show multiple sequence alignments of each ITS region of *Acholeplasma* for selecting genus-specific probes.

FIG. 3 shows a result of PCR amplification using primer pairs which can amplify ITS target sequences of many *Mycoplasma* and its related strains

FIG. 4 shows a microarray comprising probes for detecting genotypes of *Mycoplasma* and its related strains as a set on a support.

FIGS. 5a to 5k show results of image analysis of specific hybridization reaction of each probes for detecting genotypes of *Mycoplasma* and its related strains and results of numerical analysis calculated from pixel intensity.

5

Best mode for carrying out the Invention

The present invention will be described in greater detail by means of following examples. The following examples are for illustrative purpose and are not intended to limit the scope of the invention.

10

Example 1: Incubation of *Mycoplasma* and its related strains and Isolation of Genomic DNA

15

Total 25 kinds of strains, including 1 kind of *Acholeplasma*, 23 kinds of *Mycoplasma*, and 1 kind of *Ureaplasma* were obtained from the American Type Culture Collection (ATCC). The strains were cultured in each culturing media under each culturing conditions according to manual provided by ATCC. From the cultured media, strain colonies were obtained with a white gold ear and input in 1.5ml tube, 100 μ l of InstaGene matrix (Bio-Rad, USA) was added thereto and suspended, and reaction was performed at 56°C for 30 minutes in constant temperature bath. And then, the reactant was shook for 10 seconds, heated at 100°C for 8 min, shook again for 10 sec, centrifuged at 12,000 rpm for 3 min, transferred to new tube, and freeze-stored at -20°C. The product was used as template DNA of PCR reaction.

20

25

The strains used were as followed:

Acholeplasma laidlawii (ATCC 25937)

Mycoplasma arginini (ATCC 23838)

Mycoplasma arthritidis (ATCC 19611)

Mycoplasma bovis (ATCC 27368)

30

Mycoplasma cloacale (ATCC 35276)

- .
- Mycoplasma falconis* (ATCC 51372)
- Mycoplasma faecium* (ATCC 25293)
- Mycoplasma fermentans* (ATCC 19989)
- Mycoplasma genitalium* (ATCC 33530)
- 5 *Mycoplasma hominis* (ATCC 23114)
- Mycoplasma hyorhinis* (ATCC 17981)
- Mycoplasma hyosynoviae* (ATCC 25591)
- Mycoplasma muris* (ATCC 33757)
- Mycoplasma neurolyticum* (ATCC 19988)
- 10 *Mycoplasma opalescens* (ATCC 27921)
- Mycoplasma orale* (ATCC 23714)
- Mycoplasma penetrans* (ATCC 55252)
- Mycoplasma pirum* (ATCC 25960)
- Mycoplasma pneumoniae* (ATCC 15531)
- 15 *Mycoplasma primatum* (ATCC 15497)
- Mycoplasma pulmonis* (ATCC 14267)
- Mycoplasma salivarium* (ATCC 23064)
- Mycoplasma spermatophilum* (ATCC 49695)
- Mycoplasma synoviae* (ATCC 25204)
- 20 *Ureaplasma urealyticum* (ATCC 27618)

Example 2: Preparation of probes for detection of *Mycoplasma* and its related strains

The probes used for detection of *Mycoplasma* and its related strains were selected based on a result of multiple alignment of ITS sequences of *Mycoplasma*, *Acholeplasma* and *Ureaplasma*. Among *Mycoplasma* and its related species, 16S rRNA sequences has high similarity of 74~97%, whereas ITS sequences has lower similarity of 25.4~78.8% except for between *M. salivarium* and *M. hyosynoviae*, and *M. hominis* and *M. falconis*. In other words, ITS contains a region more polymorphic than 16S rRNA which is useful for designing probes for

detection of *Mycoplasma* and its related strains. However, to complement specificity between *M. salivarium* and *M. hyosynoviae*, and *M. hominis* and *M. falconis* having a high ITS similarity, more restrictive and strict probes were designed.

5 In the present invention, the oligonucleotide probes for detection of *Mycoplasma* and its related strains were prepared by synthesizing 15-25 bases of specific probe with 15 bases of dT spacer at 5' end. Probes for detection of *Mycoplasma* and its related strains are not restricted to the sequences disclosed in Tables 2 and 3 and any primer and probes comprising the sequences can be used in the present invention.

10 1. Preparation of probes for detection of *Mycoplasma* and *Ureaplasma*
15 ① Preparation of probes for genus-specific detection of *Mycoplasma* and *Ureaplasma*

20 For genus-specific hybridization with all *Mycoplasma* and *Ureaplasma* genus, probes of SEQ ID Nos. 7 and 8 in Table 2 were designed from conserved sequences of ITS of *Mycoplasma*. Further, each Group-based conserved sequences targeted to *Mycoplasma* ITS were designed as follows. For detecting Group I (*M. arginins*, *M. arthritidis*, *M. cloacale*, *M. falconis*, *M. faecium*, *M. hominis*, *M. hyosynoviae*, *M. orale*, *M. salivarium*), probes of SEQ ID Nos. 9 and 10 were designed. For detecting Group II (*M. bovis*, *M. fermentans*, *M. opalescens*, *M. primatum*, *M. spermophilum*, *M. synoviae*), probes of SEQ ID Nos. 11, 12, 13 and 14 were designed. For detecting Group III (*M. muris*, *M. penetrans*, *U. urealyticum*), probes of SEQ ID Nos. 15 and 16 were designed. For detecting Group IV (*M. neurolyticum*, *M. pulmonis*), probes of SEQ ID Nos. 17, 18 and 19 were designed. For detecting Group V (*M. genitalium*, *M. pirum*, *M. pneumoniae*), probes of SEQ ID Nos. 20 and 21 were designed.

② Preparation of probes for species-specific detection of *Mycoplasma* and *Ureaplasma*

For species-specific hybridization with each *Mycoplasma* and *Ureaplasma* species, 100 kind of probes of SEQ ID Nos. 28 to 127 in Table 3 were designed from species-specific sequences of ITS of *Mycoplasma* and *Ureaplasma*, which can detect 25 kind of *Mycoplasma* strains.

10 2. Preparation of probes for detection *Acholeplasma*

① Preparation of genus-specific probes for detection *Acholeplasma*

For genus-specific hybridization with all *Acholeplasma* genus, probes of SEQ ID No. 22 in Table 2 was designed from conserved sequences targeted to both of ITS1 and ITS2 of *Acholeplasma*. Further, each Group-based conserved sequences targeted to each *Acholeplasma* ITS1 and ITS2 were designed as follows. For Group I targeted to ITS1, probes of SEQ ID Nos. 23, 24 and 25 were designed. For Group II targeted to ITS2, probes of SEQ ID Nos. 26 and 27 were designed.

② Preparation of species-specific probes for detection *Acholeplasma*

For species-specific hybridization with each *Acholeplasma* species, probes of SEQ ID Nos. 128 to 133 in Table 3 were designed from species-specific sequences of ITS of *Acholeplasma*.

Example 3: Preparation of target DNA

1. Preparation of target DNA for detection of *Mycoplasma* and its related strains

30 For preparing target DNA for detection of *Mycoplasma* and its

related strains, 187~290bp size of ITS regions were selectively amplified using 5'-biotin-GTG(C/G)GG(A/C)TGGATCACCTCCT-3' (MP16SF-2) and 5'-biotin-GCATCCACCA(A/T)A(A/T)AC(C/T)CTT-3' (MP23SR-2), and 5'-biotin-AAAGTGGGCAATACCCAACGC-3' (M78) and 5'-biotin-
5 CCACTGTGCCCTTGTTCCT-3' (R34) which were biotin-labeled respectively (Tang et al., 2000.). To prepare genomic DNAs of *Mycoplasma* and its related strains isolated in Example 1, PCR were carried out using the above primers in the following conditions: denaturation at 94°C for 3 minutes, 30 cycles of amplification at 94°C for
10 30 seconds, at 55°C for 2 minutes and at 72°C for 2 minutes, and final extension at 72°C for 10 minutes. After the reaction, the reaction products were analyzed by ELECTROPHORESIS on a 2% agarose gel. FIG. 3 is an electrophoresis image taken after the PCR performed using primers capable to amplify ITS target sequences of several *Mycoplasma*.

15

Example 4: Probe immobilization on support

Among the probes prepared in Example 2, each representative probes for *Mycoplasma*, Acholeplasma and Ureaplasma were selected. Each of the selected probes was transferred to 384-well microplate,
20 diluted to a concentration of 50 pmole by adding spotting solution, and immobilized on a slide glass using a microarrayer (Cartesian Technologies, USA). In FIG 4, each probes for detection of *Mycoplasma* and its related strains correspond to SEQ ID Nos. 7, 28, 30, 33, 38, 41, 49, 52, 58, 61, 69, 75, 83, 85, 87, 30, 90, 92, 96, 100, 105,
25 110, 114, 120, 122, 22, 128, and 7 in order. Two spots of each kind of the probes were attached to the support and left in a slide box at room temperature for 24 hours or in a dry oven at 50°C for about 5 hours to be fixed to the surface of the support.

30

Example 5: Unimmobilized probe washing

The slide glass after the process in Example 4 was washed with

a 0.2% SDS buffer solution and then distilled water at room temperature to remove unimmobilized probes. The washed slide glass was immersed in a sodium borohydride (NaBH₄) solution for 5 minutes and then washed again at 100°C. Final washing with a 0.2% SDS solution and then distilled water was followed by centrifugation to fully dry the slide glass.

5
Example 6: Hybridization

The biotin-labeled target products prepared in Example 3 were thermally treated to be denatured into single strands and cooled to 4°C. A hybridization reaction solution containing 2 μ l of the target products was prepared. This hybridization reaction solution was portioned on the slide glass after the process in Examples 4 and 5, and the slide glass was covered with a cover slip and reacted at 25°C for 1 hours.

15

Example 7: Unhybridized tart DNA washing

TO WASH OUT UNHYBRIDIZED TARGET DNAs, THE COVER SLIP WAS REMOVED USING A 2X SSC WASHING SOLUTION (300MM NACL, 30MM NA-CITRATE, PH 7.0), AND THE SLIDE WAS WASHED WITH 2X SSC AND THEN 0.2X SSC, FOLLOWED BY CENTRIFUGATION TO FULLY DRY THE SLIDE GLASS.

20
Example 8: Staining and Result analysis

To determine hybridization of PCR products and probes, Cy5-streptavidin or Cy3-streptavidin (Amersham pharmacia biotech, USA) was diluted with 6x SSC and BSA (Bovine Serum Albumin), about 40 μ l of dilutes was portioned on slide glass, and the slide glass was covered with a cover slip to block light and reacted at 50°C for about 20 minutes. After the reaction, the cover slip was removed using a 2X SSC solution, and the slide was washed with 2X SSC and then 0.2X SSC. The

hybridized result was scanned using a non-confocal laser scanner (GenePix 4000A, Axon Instruments, U.S.A.) and analyzed by image analysis.

5 FIG. 5 shows results of image analysis of specific hybridization reaction of each probes for detecting genotypes of representative 11 kinds of *Mycoplasma* and its related strains and results of numerical analysis calculated from pixel intensity.

10 FIG. 5a shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 85) of *M. cloacale*. FIG. 5b shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 87) of *M. falconis*. FIG. 5c shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ 15 ID No. 90) of *M. hyosynoviae*. FIG. 5d shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 49) of *M. neurolyticum*. FIG. 5e shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 52) of *M. opalescens*. FIG. 5f shows results of hybridization reaction of genus-specific probe (SEQ ID 20 No. 7) and species-specific probe (SEQ ID No. 69) of *M. penetrans*. FIG. 5g shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 61) of *M. pirum*. FIG. 5h shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 83) of *M. salivarium*. FIG. 5i shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 100) of *M. spermatophilum*. FIG. 5j shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 122) of *U. urealyticum*. FIG. 5k shows results of hybridization reaction of genus-specific probe (SEQ ID No. 22) and

species-specific probe (SEQ ID No. 128) of *A. laidlawii*.

Industrial Applicability

As described above, the present invention provides a rapid and accurate assay method capable of simultaneously detecting many *Mycoplasma* and its related strains from a single sample using a microarray comprising novel oligonucleotides for detecting *Mycoplasma* and its related strains which are known as a source of contamination of cell lines and biological products and human pathogenic.

Also, the present invention provides an objective and credible assay method capable of tracing a contamination source for preventing expansion of infective *Mycoplasma* and its related strains and controlling a contamination of *Mycoplasma* against biological products and stem cells or cord blood cells which are useful for gene therapy and cell therapy.

Further, the present invention provides very specific and sensitive hybridization assay for detecting *Mycoplasma* and its related strains using oligonucleotide probes designed based on sequence analysis of ITS region of many *Mycoplasma* Strains.

20

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What is claimed is:

1. An ITS (Internal transcribed spacer) target DNA for genotyping *Mycoplasma* strains, comprising any one sequence selected from SEQ ID Nos. 1 to 6.
2. An oligonucleotide for genus-specific genotyping of *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 7 to 21 or its complementary sequence.
3. An oligonucleotide for species-specific genotyping of *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 28 to 127 or its complementary sequence.
4. An oligonucleotide for genus-specific genotyping of *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 22 to 27 or its complementary sequence.
5. An oligonucleotide for species-specific genotyping of *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 128 to 133 or its complementary sequence.
6. A microarray comprising more than one oligonucleotide selected from genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma*, *Acholeplasma* and *Ureaplasma* strains according to any one from claims 2 to 5 as probes attached on a support.
7. The microarray according to claim 6, wherein the probes are any one selected from a group consisting of DNA, RNA, PNA, LNA and HNA.
8. The microarray according to claim 6, wherein the support is any

one selected from a group consisting of slide glass, plastic, membrane, semiconductive chip, silicon and gel.

9. A method for detecting *Mycoplasma* strains, comprising the
5 following steps:

- a) extracting nucleic acids from a sample;
- b) amplifying target DNA among the extracted nucleic acids;
- c) hybridizing the amplified target DNA with probes of the microarray according to claim 6; and
- 10 d) detecting signals generated from the hybridization reaction.

10. A kit for diagnosing *Mycoplasma* infection, comprising more than one oligonucleotide selected from genus-specific and species-specific oligonucleotides for genotyping *Acholeplasma*, *Mycoplasma* and
15 *Ureaplasma* strains according to any one from claims 2 to 5.

1/10

FIG. 1a

| | | | |
|------------------------------------|--|---|-----|
| <i>M. bovis</i> | -----ATA----- | TGTTCTTGTAAAACGTGAAATAGTAAAATATT----- | 142 |
| <i>M. primigenius</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATAGTAAAATATT----- | 181 |
| <i>M. fermentans</i> | -----ATT----- | TGTTCTTGTAAAACGTGAAATAGTAAAATATT----- | 177 |
| <i>M. leprae</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATAGTAAAATATT----- | 159 |
| <i>M. avium</i> ssp. | -----AT----- | TGTTCTTGTAAAACGTGAAATAGTAAAATATT----- | 196 |
| <i>M. avium</i> ssp. | -----AAI----- | TTTCTTGTAAAACGTGAAATAGTAAAATATT----- | 100 |
| <i>M. avium</i> ssp. | TAATAAATGTTT----- | AATATTCTTGTAAAACGTGAAATAGCATA---TA---T | 176 |
| <i>M. pulmonis</i> | -AACATA----- | TTTCTTGTAAAACGTGAAATAGCATA---TAAT----- | 159 |
| <i>M. hyoileum</i> | -----ATA----- | TTTCTTGTAAAACGTGAAATAGCATA-----TAA----- | 112 |
| <i>M. avium</i> ssp. <i>filiis</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATA-----T-----T | 115 |
| <i>M. Faeni</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATA-----T-----T | 123 |
| <i>M. orale</i> | -----II----- | TAATCTTGTAAAACGTGAAATA-----I-----I | 108 |
| <i>M. tuberculosis</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATA-----T-----T | 119 |
| <i>M. scrofulaceum</i> | -----TT----- | TGTTCTTGTAAAACGTGAAAT-----T-----T | 115 |
| <i>M. falciante</i> | -----TT----- | TGTTCTTGTAAAACGTGAAAT-----T-----T | 92 |
| <i>M. tuberculosis</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATA-----T-----T | 107 |
| <i>M. arginini</i> | -----TT----- | TGTTCTTGTAAAACGTGAAATA-----T-----T | 93 |
| <i>M. celatum</i> | -----II----- | TAATCTTGTAAAACGTGAAATA-----I-----I | 96 |
| <i>M. genitalium</i> | CGAGTCGTAAAB-AATTTTTTGTAAACGTCCTTCAAAAACGTGAAAGGACA----- | | 160 |
| <i>M. pneumoniae</i> | CCAGTTCTGAAAG-AACATTTCGCG----- | TCTTCTTGTAAAACGTGAAAGGACA----- | 190 |
| <i>M. pirum</i> | TAATTTTTTGAGTAGTAGAGATGG----- | TCTTGTAAAACGTGAAAGGACA----- | 213 |
| <i>M. muscae</i> | TT----- | CTTGTAAAACGTGAAATACTGATA----- | 100 |
| <i>M. parvum</i> | TT----- | CTTGTAAAACGTGAAATTTATAAA----- | 184 |
| <i>M. urealyticum</i> | TTAATTTATTAAG-----GATGATGA----- | TCTTGTAAAACGTGAAATAI-----I-----I | 199 |

FIG. 1b

| | | |
|-----------------------|----------|---|
| <i>S. enteritidis</i> | AAA— | CAT TGT ATG CAG I TTT GAG AGA A C T T A A A C T T C G T C G T T T G T T C T T G A A A A C — 108 |
| <i>S. Braenderup</i> | AAA— | CAT C G T ATG CAG I TTT GAG AGA A C T T A A C T T C G T C G T T T G T T C T T G A A A A C — 68 |
| <i>S. Typhimurium</i> | TAA— | A T T T G T A T G C A G I T T T G A G A G A C T T A — A C T T G T T T T T G T T C T T G A A A A C — 65 |
| <i>S. Infantis</i> | AAAAAA | A T T T G T A T G C A G I T T T G A G A G A C T T A — T C T G T G G — G T T C T T G A A A A C — 50 |
| <i>S. Agona</i> | AAA—T | A T T T G T A T G C A G I T T T G A G A G A C T T A — T C T G T G A A T T T G T T C T T G A A A A C — 56 |
| <i>S. Enteritidis</i> | CAA TT A | A T T T G T A T G C A G I T T T G A G A G A C T T — T C T G T G A A T T T G T T C T T G A A A A C — 88 |
| <i>S. Infantis</i> | CA— | A T T C G T A T G C A G I T T T G A G A G A C T T A T T G T C T G T T T T T T G T T C T T G A A A A C — 113 |
| <i>S. Braenderup</i> | CAA—T | A T T C G T A T G C A G I T T T G A G A G A C T T A T T G T C T G T T T T T T G T T C T T G A A A A C — 102 |

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| | | | |
|------------------------|--------------------------|----------------------------|-----|
| <i>S. arthritidis</i> | —TTAAAAAAATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATACAA | 173 |
| <i>S. taucium</i> | —TTAAAAAAATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATACAA | 153 |
| <i>S. falconis</i> | ATTA—TTAATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATACAA | 150 |
| <i>S. hominis</i> | —TA—TTAATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATAATT | 141 |
| <i>S. arginini</i> | ATTAATTATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATAAT | 153 |
| <i>S. cincinnati</i> | —TCATTAATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATAATT | 154 |
| <i>S. hyoilemoniae</i> | A—TTATCAAATTAAATATTCAAA— | GTTTAGATGCAACCTATAGAATAATT | 178 |
| <i>S. urale</i> | —TTAAAATTAAATATTCAAAA— | TTTAGATGCAACCTATAGAATAATT | 166 |

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FIG. 1c

| | | | | | |
|---------------------------|-------------------------------------|---|------------------------|-----------------|-----|
| <i>S. bovis</i> | TTATTATAGGTCAGAAGCTA | XIXCTCTTGTGAGAGCTA | TTCTCTCT 144 | | |
| <i>H. primateus</i> | TT---TAATGGGTGGAGCTT | ATATCTCTTGTGAGAGCTA | TTCTCTCTT 148 | | |
| <i>H. fermeinata</i> | TITTTATGGGTCAAAGCTT | ATATCTCTTGTGAGAGCTAATACTTTTCTCTAT | TTCTCTCTT 146 | | |
| <i>H. opalescens</i> | T-----ATGGTCTACAAAGCT | ATATCTCTTGTGAGAGCTA | TTCTCTCTT 129 | | |
| <i>H. spumiferophytum</i> | TT---TTATGGGCTAAAGCTT | ATATCTCTTGTGAGAGCTA | TCTCTCTATA 166 | | |
| <i>H. syriacus</i> | GGTTTTTGCGCTGGGCTAT | ATATCTCTTGTGAGAGCTA | CCTCTCTAAAG 141 | | |
| | *** | *** | *** | | |
| <i>S. bovis</i> | ATGTTCTTGTGAAACTGAATACTAAAATATTTT | ATATCTCTTGTGAGAGCTAATAAA | 201 | | |
| <i>H. primateus</i> | -TGTCTTGTGAAACTGAATACTAAAATATTTT | ATATCTCTTGTGAGAGCTAATAAA | 207 | | |
| <i>H. fermeinata</i> | TTGTTCTTGTGAAACTGAATACTAAA | ATATCTCTTGTGAGAGCTAATAAA | 200 | | |
| <i>H. opalescens</i> | -TGTCTTGTGAAACTGAATACTAAA | ATATCTCTTGTGAGAGCTAATAAA | 182 | | |
| <i>H. spumiferophytum</i> | -TGTCTTGTGAAACTGAATACTAAA | ATATCTCTTGTGAGAGCTAATAAA | 219 | | |
| <i>H. syriacus</i> | TGTTCTTGTGAAACTGAATACTAAA | TAAGGATATTACAAAGCTATAAAAT | 198 | | |
| | *** | *** | * | | |
| <i>S. bovis</i> | -ATCAA-----TGTCTTGTGAGAGCTAATTTA | -AATGCTATATTTA | 250 | | |
| <i>H. primateus</i> | CCATCAAAATTTAATGGCTTGTGAGAGCTAATTTA | -AATGCTATATTTA | 261 | | |
| <i>H. fermeinata</i> | TTAAA-----TGTCTTGTGAGAGCTAATTTA | -AATGCTATATTTA | 250 | | |
| <i>H. opalescens</i> | -ATTAATTGATTAA | GGCTCAATTGTGAGAGCTAATTTA | TAAGGATATAACATCATATAAA | | |
| <i>H. spumiferophytum</i> | TAATTGAA-----TGTCTTGTGAGAGCTAATTTA | TCATGAGATATAAAACATCATATAAA | 238 | | |
| <i>H. syriacus</i> | -ATATTTAATTAA | GGCTCAATTGTGAGAGCTAATTTA | TATGCTATTTATAAA | 270 | |
| | *** | *** | * | | |
| <i>S. bovis</i> | -TATGATCATTGAAATGCTT | TAATACACATCTAAA | ACTAACACATAAGG | 304 | |
| <i>H. primateus</i> | -TATGATCATTGAAATGCTT | AAATACACATCTAAA | ACTAAACAACTAGG | 313 | |
| <i>H. fermeinata</i> | -TATGATCATTGAAATGCTT | AAATACACATCTAAA | AACCTATAACATAAGG | 306 | |
| <i>H. opalescens</i> | TTTTGAACTGAAATGCTT | AAATACACATCTAAAATACATDATAATGT | AACGAAATACAATAAGG | 298 | |
| <i>H. spumiferophytum</i> | AAATGATCATTGAAATGCTT | AAATACACATCTAAAATACATCTAAACAAACATCTATAACAATAAGG | 290 | | |
| <i>H. syriacus</i> | -TATGATCATTGAAATGCTT | TAATACAA | TCATAAC | AAATATAACATAAGG | 295 |
| | *** | *** | *** | | |

FIG. 1d

| | | | | |
|------------------------|---------------------------------------|---------------------------------|---------------------|-----|
| <i>H. suris</i> | CGCTCTTCTATCGGCTTACATTATTTAATT | TTTACACCAATTAGCTTAA | TTTACACCAATTAGCTTAA | 60 |
| <i>H. parvirostris</i> | CGCTCTTCTATCGGCTTACATTAAAGCTTAA | TTTACACCAATTAGCTTAA | ACTAAACAACTAGG | 51 |
| <i>H. urivalicus</i> | CGCTCTTCTATCGGCTTAAATTTAATT | TTTACACCAATTAGCTTAA | TTTACACCAATTAGCTTAA | 53 |
| | *** | *** | *** | |
| <i>H. suris</i> | TGTGTACTTT-TTATGAAACCGGCTCATATAACCTAA | -----ATAAACATTATTT | 115 | |
| <i>H. parvirostris</i> | TGTGTACTTTAATGAAACCGGCTCATATAACCTAA | -----TATGCTCAIGTAAATATAAGCTAAGT | 112 | |
| <i>H. urivalicus</i> | TATTAATGAAACCGGCTCATATAACCTTTTAAATTT | -----TMAAATTCTATAT | 103 | |
| | *** | *** | *** | |
| <i>H. suris</i> | TTTGCGCGGATCTATTTGAGCTT | TTTCTCTCTATGAGCTT | ----- | 165 |
| <i>H. parvirostris</i> | TCAGGCGGATCTATTTGAGCTT | TTTCTCTCTATGAGCTT | ----- | 162 |
| <i>H. urivalicus</i> | --CGCGGATCTATTTGAGCTT | TTTCTCTCTATGAGCTT | -----TAACTTAACTTAT | 165 |
| | *** | *** | *** | |

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FIG. 1e

FIG. If

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FIG. 2a

| | | |
|-------------------------|---|-----|
| <i>A. aculeatus</i> | AAACATTCCTGATTTGTCATCATATTAGTTTTCAGAAGCTT----- | 88 |
| <i>A. aculeatus</i> | AAACATTCCTGATTTGTCATCATATTAGTTTTCAGAAGCTT----- | 110 |
| <i>A. ferdinandi</i> | TAACTTCTCTAAATTGTCATCATATTAGTTTTCAGAAGCTT----- | 103 |
| <i>A. ferdinandi</i> | TAACTTCTCTAAATTGTCATCATATTAGTTTTCAGAAGCTT----- | 96 |
| <i>A. mediterraneus</i> | TTAACATTCATATGATTTCATCATATTAGTTTTCAGAAGCTT----- | 73 |

FIG. 2b

| | | |
|-------------------------|---|-----|
| <i>A. ferdinandi</i> | CAAGTAACCACTATAATAAA&AGTGGCGCTGAACTGAGCTGCTT----- | 169 |
| <i>A. aculeatus</i> | CAA-----AGTGGCGCTGAACTGAGCTGCTT----- | 156 |
| <i>A. acanthopus</i> | TAA-----TAAAGGGCGCTGAACTGAGCTGCTT----- | 133 |
| <i>A. acanthopus</i> | TAA-----TGGCGCTGAACTGAGCTGCTT----- | 122 |
| <i>A. ferdinandi</i> | TGATTAACCGCTGCTTCAAGCTGCTT----- | 227 |
| <i>A. aculeatus</i> | TGTAAACCGCTGCTTCAAGCTGCTT----- | 201 |
| <i>A. acanthopus</i> | TGTAAACCGCTGCTTCAAGCTGCTT----- | 184 |
| <i>A. mediterraneus</i> | TGTAAACCGCTGCTTCAAGCTGCTT----- | 172 |

FIG. 2c

| | | |
|-------------------------|--|-----|
| <i>A. ferdinandi</i> | GAA---TATTCCTAAATTTGTCATCATATTAGTTTGAAAGCTAA---AGAAATT----- | 104 |
| <i>A. aculeatus</i> | GAAAGCTTCTAAATTTGTCATCATATTAGTTTGAAAGCTAA---TGCAGCTG----- | 116 |
| <i>A. acanthopus</i> | -----AAACATTTCTCAATTTGTCATCATATTAGTTTGAAAGCTTTG---AGCTGTT----- | 96 |
| <i>A. mediterraneus</i> | -----CATTTTCATCATATTAGTTTGAAAGCTTTGCTTCTGAT----- | 84 |
| <i>A. ferdinandi</i> | -----TAACTTCTAAAGAAAGCTTTGAAAGCTAAAGAAAGAA-----GATGCTGAAA----- | 102 |
| <i>A. aculeatus</i> | -----TTCGTTCTAAAGCTTCATCATATTAGCTTTGAAAGCTAAAGAA-----GATGCTGAAA----- | 112 |
| <i>A. acanthopus</i> | -----C---TCAG-----AAGCTTCATCATATTAGCTTTGAAAGCTAAAGAA-----GAAGTCGAAA----- | 102 |
| <i>A. mediterraneus</i> | -----AGTAAAGCTTTGAAAGCTTCATCATATTCTGCTGAA-CAGAAAT-----TAAAGAGAGAA----- | 146 |

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FIG. 3a

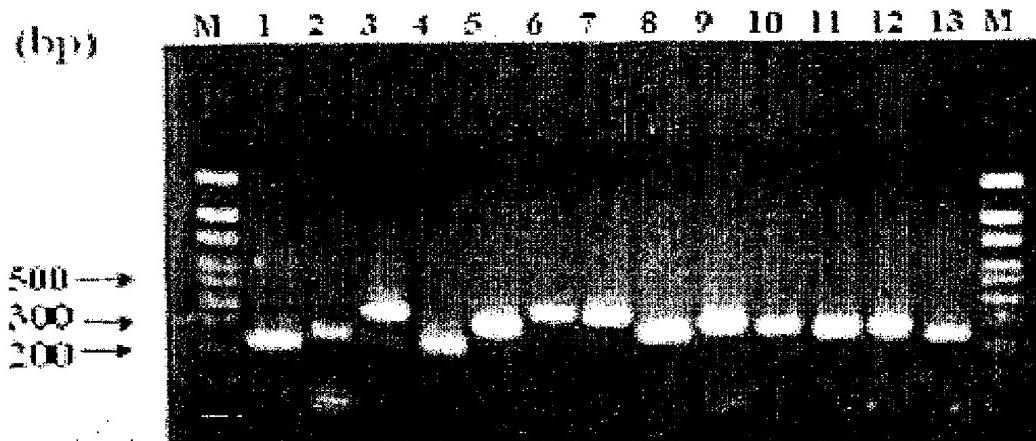
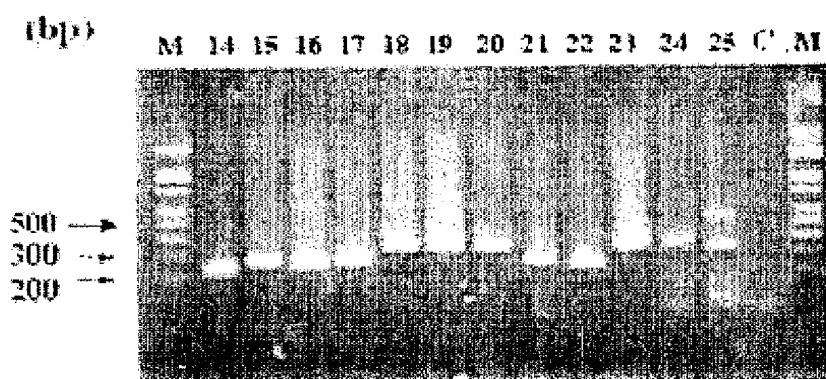


FIG. 3b



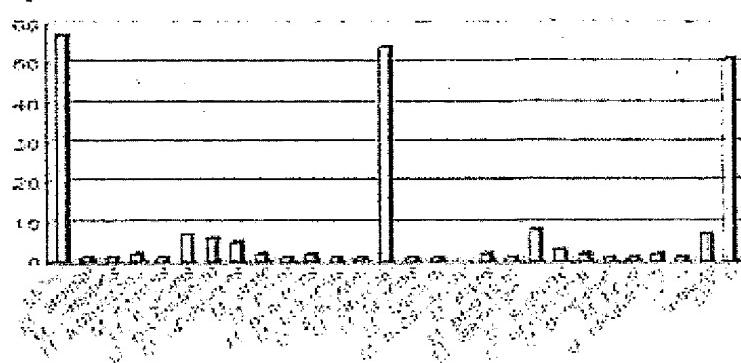
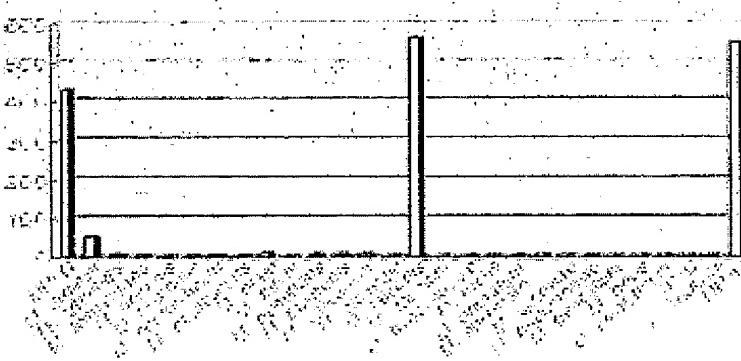
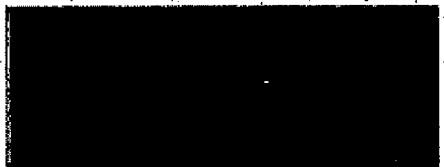
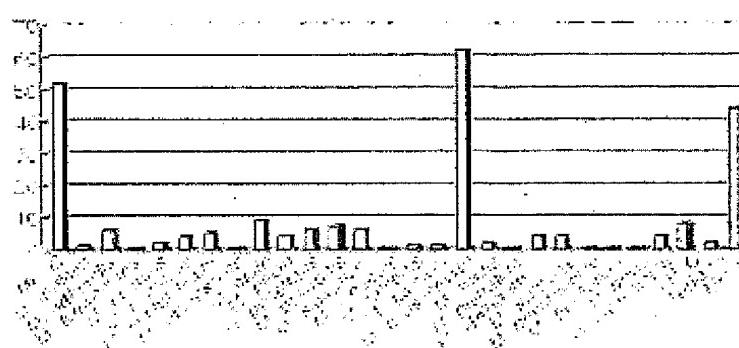
6/10

FIG. 4

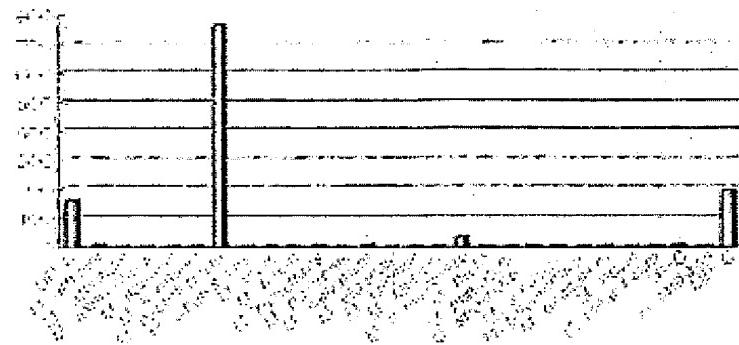
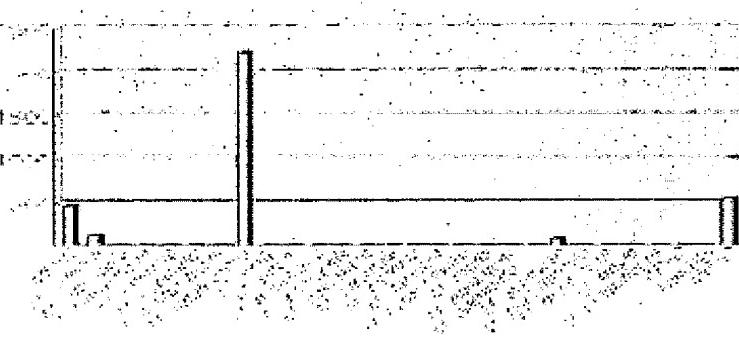
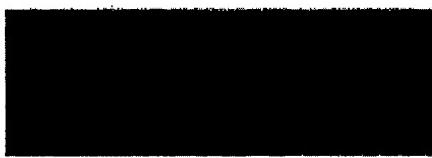
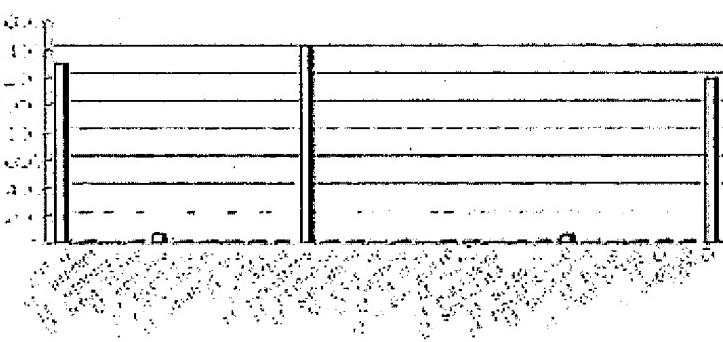
| MP-C [7] | <i>M. arginini</i> [28] | <i>M. artibidis</i> [30] | <i>M. fermentans</i> [93] | <i>M. hominis</i> [38] | <i>M. hyorhinis</i> [41] |
|--------------------------------|---------------------------------|-----------------------------|-------------------------------|-------------------------------|-----------------------------|
| <i>M. neovarivorum</i> [49] | <i>M. opalescens</i> [52] | <i>M. orale</i> [58] | <i>M. pirum</i> [61] | <i>M. penetrans</i> [69] | <i>M. pulmonis</i> [75] |
| <i>M. salivarium</i> [83] | <i>M. citacae</i> [85] | <i>M. falconis</i> [87] | <i>M. faecium</i> [30] | <i>M. hyosynoviae</i> [90] | <i>M. muris</i> [92] |
| <i>M. primatum</i> [96] | <i>M. spermophilum</i> [100] | <i>M. synoviae</i> [105] | <i>M. pneumoniae</i> [110] | <i>M. genitalium</i> [114] | <i>M. bovis</i> [120] |
| <i>U. urealyticum</i> [122] | | | AP-C [22] | <i>A. laidlawii</i> [128] | MP-C [7] |

*[] corresponds to SEQ ID No's of Tables 2 and 3.

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FIG. 5a**FIG. 5b****FIG. 5c**

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FIG. 5d**FIG. 5e****FIG. 5f**

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FIG. 5g

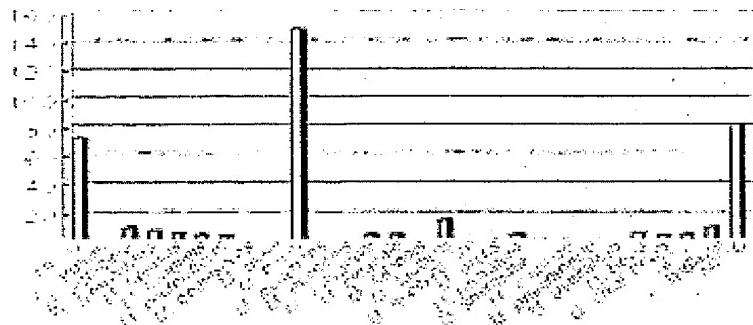


FIG. 5h

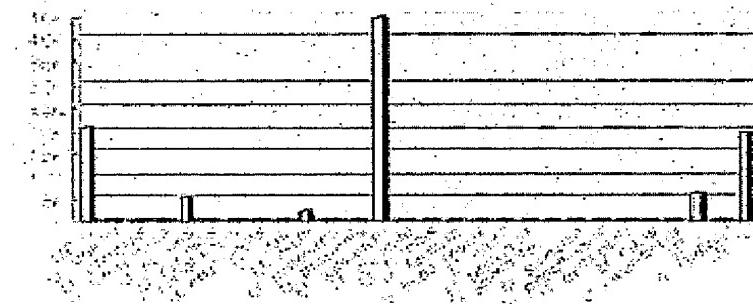
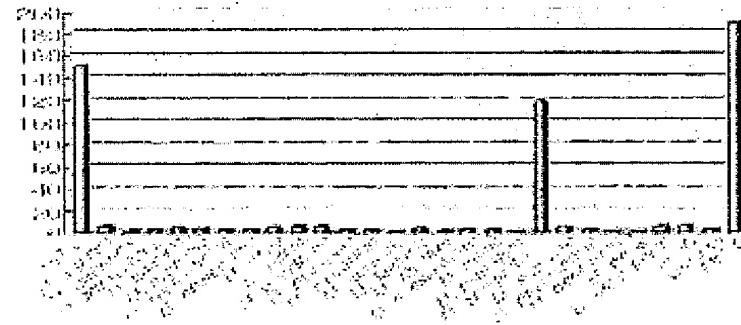
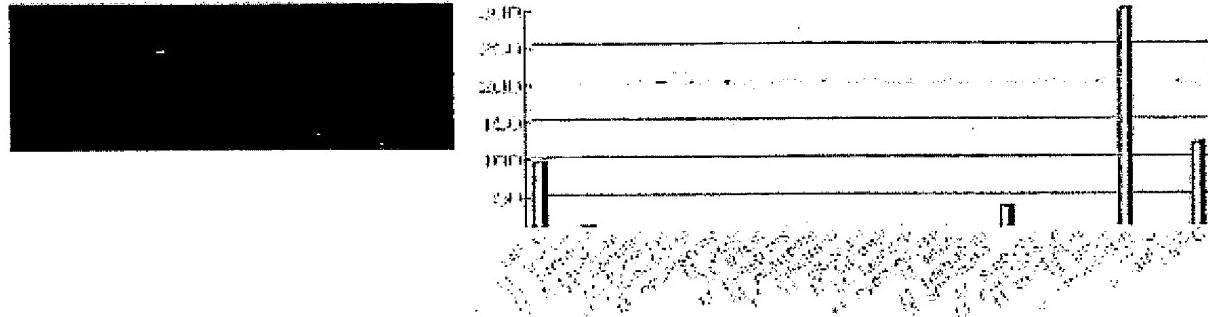
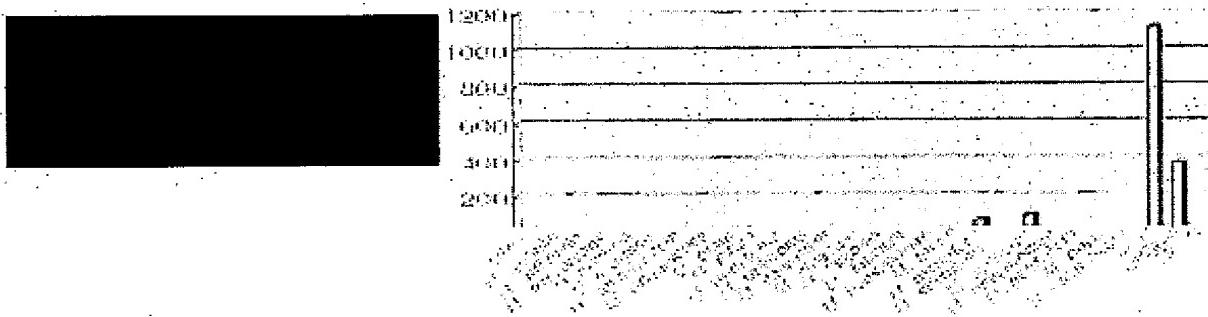


FIG. 5i



10/10**FIG. 5j****FIG. 5k**

SEQUENCE LISTING

<110> GENEIN CO., LTD.
KIM, Cheol-Min
PARK, Hee-Kyung

<120> Oligonucleotide for genotyping of *Mycoplasma*, microarray comprising the oligonucleotide, and method for detection of species using the microarray

<130> PN053079

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agttttgaga aaacat tctc tcataatgttc ttgtaaaact gaatagtaaa atattttgc 180

atatttacaa ccgacatcaa aatcaaatta atggtaatt tttttgtatt catcgactaa 240

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<212> DNA

<213> M. cloacale

<400> 2

| | |
|--|-----|
| cttctacgga gtacaattct cactgttatg gaattaaatt tgtatccagt tttgagagaa | 60 |
| ctttctctca attttgtct ttgaaaactg aatatagaca ttgaaatcaa taaattaata | 120 |
| tttcaaatgt tttagatcaac ctatagaata ttcagaacat atacaaaaat aggtcatact | 180 |
| tatatttata aatact | 196 |

<210> 3

<211> 196

<212> DNA

<213> M. falconis

<400> 3

| | |
|---|-----|
| ctttctacgg agtacaacctt ctgttatgga ataataatttg tatccagttt tgagagtact | 60 |
| aacictcttt ttgttccttg aaaactgaat atcgacattt gaaaattttt aatttaattt | 120 |
| tcaaagtta gaicaaccta tagaatacaa aaatatagac aacaaiaggt catacaacaa | 180 |
| acataacaaa acaact | 196 |

<210> 4

<211> 239

<212> DNA

<213> M. faecium

<400> 4

| | |
|--|-----|
| gaatggtggc ttcgagacta aaagttatgg aaaaacatcg tatccagttt tgagagaact | 60 |
| aaacttctctt ctttgttct ttgaaaactg aatatagaca tigaaaatta aaaaattaat | 120 |
| atttcaaagt tttagatcaac ctatagaata caaaatcaat acaataggtc aatactatac | 180 |
| aattgcataa caaaaaataac tattaaacaa gataagagtt tttggggat gcaattgta | 239 |

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<211> 340
<212> DNA
<213> M. spermatophilum

<400> 5
gtggggatgg atcacctctt ttcacggag tacaaacata cattcaaatt ttgactgaat 60
gttatttaacc ttatttttc actaggcctt tttaatatat tttgttatgt gactttatg 120
gcctaaaagt cttataatcta gtttgagag gacatcctct ctaatgttc ttgaaaact 180
gaatagtaaa tttttgata tttacaacga catctaaataa attgaattaa gtcaatttg 240
tttagatttca tcgagatagt cattttaaaa aaatgattca ttgaaatgtc taaaataca 300
catcaaaaaca aacaatctat acaataggaa tttataiact 340

<210> 6
<211> 322
<212> DNA
<213> M. synoviae

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tcottacgga giacattaat tttacaaaag gcattttat taactgaaag cttttagaga 60
aaaattctaa aagcggttgt gtatcgcttt tttgccttg ggctattgtt tttgttttg 120
agagaacaac ctctcttaaa attgttcttt gaaaactaaa tagtaataaa gatattacaa 180
cgacatcaaa aatataaatt aattaaggtt aatttgtttt gataccgagt ttaaattatt 240
gaataataat ttataaaat gtcttgaat acaicataac aatataacaa taggacat 300
tgatactaac ttttaaaaaa gt 322

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<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Mycoplasma

<400> 7
ttctttgaaa actga 15

<210> 8
<211> 20
<212> DNA
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<220>
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<400> 8
rwtcttivaa aactrratwn 20

<210> 9
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. arginini, etc.

<400> 9
mwtygtrtcc agtttgaga g 21

<210> 10
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. arginini, etc.

<400> 10

tttagatcaa cctatagaat a

21

<210> 11
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. bovis, etc.

<400> 11

rattytagtt ttgagagrrc a

21

<210> 12
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. bovis, etc.

<400> 12

wwtrattyat traaatgtct t

21

<210> 13
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. bovis, etc.

<400> 13
ggkyaatttg titwgat

17

<210> 14
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. bovis, etc.

<400> 14
ratatttaca mcgmcayc

18

<210> 15
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. muris, etc.

<400> 15
cctcctttct atcgagatam a

21

<210> 16
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. muris*, etc.

<400> 16

cggattctat ttagtttga g

21

<210> 17
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. neurolyticum*, etc.

<400> 17

taaaat~~a~~agat acc~~t~~taakat a

21

<210> 18
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. neurolyticum*, etc.

<400> 18

gtatyy~~a~~gtt ttgaaag

17

<210> 19
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. neurolyticum*, etc.

<400> 19
ct tgccaaawt agwtwti

16

<210> 20
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. genitalium*, etc.

<400> 20
awacraacaat ctttctagtt c

21

<210> 21
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. genitalium*, etc.

<400> 21

aaaataggtaac taagggtta t

21

<210> 22
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Acholeplasma

<400> 22
tcatcaiaatt cagtttgc

18

<210> 23
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Acholeplasma

<400> 23
gggccirtag ctcaagygtt t

21

<210> 24
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Acholeplasma

<400> 24
agagcrrcwccg cytgataagg g

21

<210> 25
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Acholeplasma

<400> 25

wgrrggtcgat ggttcragtc c

21

<210> 26
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Acholeplasma

<400> 26

tcatcataatt cagttttgar r

21

<210> 27
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting Acholeplasma

<400> 27

agtctttigaa aagttagataa a

21

<210> 28
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. arginini

<400> 28

agattatatac atacaataga

20

<210> 29
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. arginini

<400> 29

gagtacataaa atgttatgga a

21

<210> 30
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. arthritidis-faucium

<400> 30

tgaaggcccgta tggtggttc g

21

<210> 31
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. arthritidis-faucium*

<400> 31
tgagagaact aaaccttctc c

21

<210> 32
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. arthritidis-faucium*

<400> 32
gaatacaaaaa tcaataacaat a

21

<210> 33
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting *M. fermentans*

<400> 33
atgtactatt aacttatttc ac

22

<210> 34
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. fermentans

1

<400> 34

tacaaaagag tactttitaa a

21

<210> 35
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

<223> Probe for detecting M. fermentans

<400> 35

ttttatggg tctaaagctt t

21

<210> 36
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

<223> Probe for detecting M. fermentans

<400> 36

gaacaatatt tttttctctc a

21

<210> 37
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. fermentans

<400> 37

ataacaaaact ataacaatag g

21

<210> 38
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe for detecting M. hominis

<400> 38

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19

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21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/000147

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 15/31, C12Q 1/68, C12Q 1/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C12N 15/31, C12Q 1/68, C12Q 1/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions since 1975Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKIPASS, Delphion, NCBI PubMed, GenBank

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| X | F. KONG, ET. AL. "Species-Specific PCR for Identification of Common Contaminant Mollicutes in Cell Culture." In: Applied and Environmental Microbiology, (July 2001) Vol.67(7):3195-3200, see the whole document. | 1 |
| X | US 5693467 (THE AMERICAN TYPE CULTURE COLLECTION) 2 December 1997 (1997-12-02) See the whole document. | 1 |
| X | R. HARASAWA, ET. AL. "boxA'-like sequence between the 16S/23S spacer in rRNA operon of mycoplasmas." In: FEBS Letters, (February 1992) Vol.297(3):209-211, see the whole document. | 1 |

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search
13 JUNE 2005 (13.06.2005)

Date of mailing of the international search report

14 JUNE 2005 (14.06.2005)

Name and mailing address of the ISA/KR


Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

AHN, Kyu Jeong

Telephone No. 82-42-481-5026



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2005/000147

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

a. type of material

- a sequence listing
 table(s) related to the sequence listing

b. format of material

- in written format
 in computer readable form

c. time of filing/furnishing

- contained in the international application as filed
 filed together with the international application in computer readable form
 furnished subsequently to this Authority for the purposes of search

2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2005/000147

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Each sequence 1 to 6, and probes for each species-specific considered to be distinct inventions since a species-specific sequence itself is an advanced technical feature for the present application.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2005/000147

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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